Compensatory Proliferation Induced by Cell Death in the *Drosophila* Wing Disc Requires Activity of the Apical Cell Death Caspase Dronc in a Nonapoptotic Role

Jun R. Huh, Ming Guo, and Bruce A. Hay

Supplemental Experimental Procedures

Fly Strains and Constructs

All crosses and stocks were maintained at 25°C. The following fly stocks were used: w^{1118}, En-Gal4, UAS-Hid, UAS-P35 [S1], UAS-Dronc^{C318S} [S2], UAS-Hid^{Ala5} (in which six MAPK phosphorylation sites in Hid are mutated to alanine) [S3], and UAS-Dronc #80 [S4]. ptc-Ga4, Act5C<CD2<Gal4, and HS-FLP were from the Bloomington Stock Center. In the UAS-Dronc #80 construct, GFP is present as a C-terminal fusion to Dronc [S4]. To generate double-stranded RNA corresponding to the coding region of DIAP1, we amplified a 605 bp fragment of DIAP1 by using primers 5′-EcoR1-GAA CAG CAC GCT CTC TGG CTA AG-3′ and 5′-Xho1-BamH1-TTT GAG GAC TTG GGT GCG CAT TGG-3′. This fragment was ligated into the EcoR1 and Xho1 sites of the SympUAST-w vector [S5], generating UAS-DIAP1-RNAi. Germ line transformants were created via standard procedures.

Generation of Clones

Larvae of genotype HS::FLP; UAS::p35; Act5C<CD2<Gal4, or HS::FLP; UAS::p35, UAS::Hid^{Ala5}; Act5C<CD2<Gal4 were heat shocked for 15 min at 37°C. Discs from third-instar larvae were processed as described below.

Immunocytochemistry

Conditions for immunocytochemistry and confocal microscopy were as described in [S1, S6]. Antibodies were used at the following concentrations: purified rabbit anti-Dronc (1:50) [S7], mouse anti-DIAP1 (1:400) [S1], mouse anti-P35 (1:100) [S1], rabbit anti-P35 (1:1,000) (Biocarta); rabbit anti-Hid (1:1,000) [S1], mouse anti-Wingless (1:30) (Developmental Studies Hybridoma Bank), and mouse anti-Phospho Histone (1:50) (Cell Siginaling Technology).

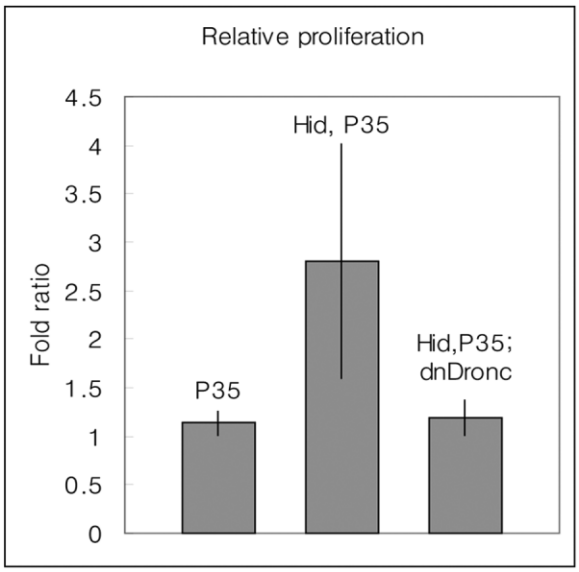

Figure S1. Wing Discs in which Hid Is Expressed in the Posterior Compartment Show a Dronc-Dependent Increase in Phosphohistone-H3 Labeling, Indicative of Increased Proliferation

The ratio of phosphohistone-H3-positive cells in the posterior versus anterior compartment (P/A ratio) was determined for discs of three different genotypes: UAS::p35/en::Gal4 (n = 4), UAS::Hid,UAS::p35/en::Gal4 (n = 3), and UAS::Dronc^{C318S};UAS::Hid,UAS::p35/en::Gal4 (n = 3).

Supplemental References

S1. Yoo, S.J., Huh, J.R., Muro, I., Yu, H., Wang, S.L., Feldman, R.M.R., Clem, R.J., Muller, H.-A.J., and Hay, B.A. (2002). Apoptosis inducers Hid, Rpr and Grim negatively regulate levels of the caspase inhibitor DIAP1 by distinct mechanisms. Nat. Cell Biol. *4*, 416–424.

S2. Hawkins, C.J., Yoo, S.J., Peterson, E.P., Wang, S.L., Vernooy, S.Y., and Hay, B.A. (2000). The *Drosophila* caspase DRONC

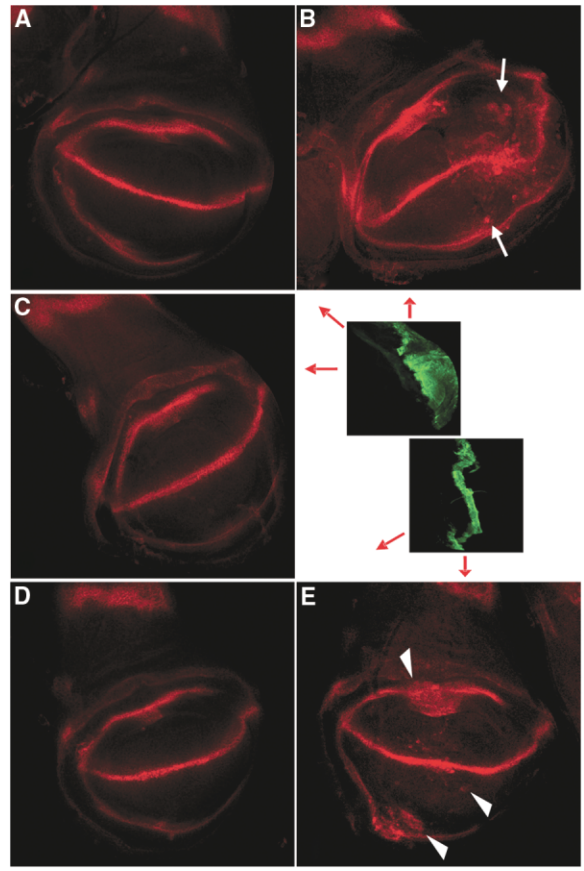

Figure S2. Wingless Levels Are Regulated by Cell Death Signaling

Confocal images of wing discs from third-instar larvae of various genotypes. All discs are stained with anti-Wingless (red). (A) Wing disc of genotype UAS::p35/en::Gal4. (B) Wing disc of genotype UAS::Hid,UAS::p35/en::Gal4. (C) Wing disc of genotype UAS::Dronc^{C318S}; UAS::Hid, UAS::p35/en::Gal4. (D) Wing disc of genotype UAS::DIAP1-RNAi;UAS::p35/ptc::Gal4. (E) Wing disc of genotype UAS::Dronc;UAS::p35/ptc::Gal4. Wingless levels in the posterior compartment are increased in the presence of Hid and p35 (B) but not p35 alone (A). This increase requires Dronc activity (C). Loss of DIAP1 alone, in the presence of p35, does not result in an increase in Wingless levels (D), whereas expression of Dronc with p35 does (E).

cleaves following glutamate or aspartate and is regulated by DIAP1, HID, and GRIM. J. Biol. Chem. *275*, 27084–27093.

S3. Bergmann, A., Tugentman, M., Shilo, B.-Z., and Steller, H. (2002). Regulation of cell number by MAPK-dependent control of apoptosis: a mechanism for trophic survival signaling. Dev. Cell *2*, 159–170.

S4. Quinn, L.M., Dorstyn, L., Mills, K., Colussi, P.A., Chen, P., Coombe, M., Abrams, J., and Kumar, S. (2000). An essential role for the caspase dronc in developmentally programmed cell death in *Drosophila*. J. Biol. Chem. *275*, 40416–40424.

S5. Giordano, E., Rendina, R., Peluso, I., and Furia, M. (2002). RNAi Triggered by Symmetrically Transcribed Transgenes in *Drosophila melanogaster*. Genetics *160*, 637–648.

S6. Wang, S.L., Hawkins, C.J., Yoo, S.J., Muller, H.A., and Hay, B.A. (1999). The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. Cell *98*, 453–463.

S7. Huh, J.R., Vernooy, S.Y., Yu, H., Yan, N., Shi, Y., Guo, M., and Hay, B.A. (2004). Multiple apoptotic caspase caspase cascades are required in nonapoptotic roles for *Drosophila* spermatid individualization. PLoS Biol 2(1): e15 DOI:10.1371/journal.pbio.0020015.